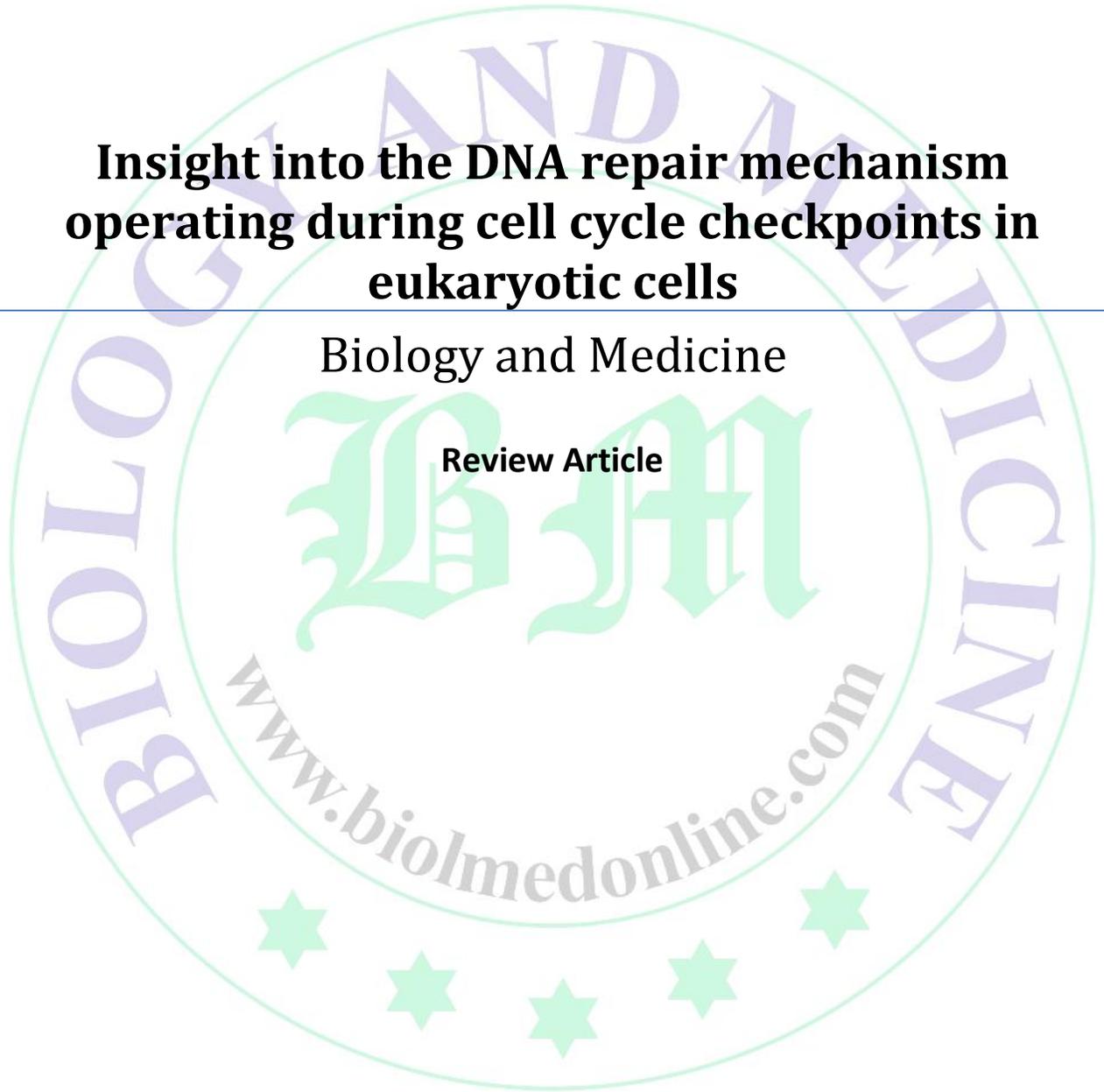


eISSN: 09748369

**Insight into the DNA repair mechanism
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Biology and Medicine

Review Article



www.biolmedonline.com

Insight into the DNA repair mechanism operating during cell cycle checkpoints in eukaryotic cells

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Accepted: 11th Sep 2012, Published: 28th Sep 2012

Abstract

In this article, we have briefly reviewed the molecular mechanism involved in DNA damage and repair at various checkpoints of the cell cycle in eukaryotic cells. Eukaryotic cells have an ability to develop a response to DNA damage that can be caused by environmental factors such as chemicals, xenobiotics, free radicals, ionizing radiation (IR) or products of intracellular metabolism and also due to products of medical therapy. In response to these insults, the following reactions can take place in the cellular environment: (a) In the case of DNA damage, the protein machinery is activated and attaches itself to the site of the lesion which results in cell cycle arrest at the G1 to S phase (the G1/S checkpoint), DNA replication phase (the intra-S checkpoint), or G2 to mitosis phase (the G2/M checkpoint) until the lesion undergoes repair; (b) As there are many different lesions possible, the following DNA repair mechanisms are activated which include direct repair, base excision repair, nucleotide excision repair, mismatch repair, and double strand breaks (DSBs) such as, homologous recombination (HR) and non-homologous end-joining (NHEJ) repair. In HR, the most important emerging proteins are the tumor suppressor proteins BRCA1, BRCA2, and Rad51 which play an important role in maintaining the genomic integrity by protecting cells from double strand breaks. Furthermore, interaction of Rad51 with BRCA2 protein complexes are essential for HR, which can be visualized by microscope as a foci and are thought to be representative sites where repair mechanism can take place. In addition, we have given special focus to the recent finding in the interaction of Rad51 with BRCA2 protein in double strand breaks by HR.

Keywords: Cell cycle checkpoints; DNA repair; double strand breaks; homologous recombination; non-homologous end-joining; BRCA1-2; Rad51.

Introduction

In everyday life, 1000 to 100,000 DNA damage incidences occur in the cell per day (Lodish *et al.*, 2004). A wide variety of damages are caused by environmental agents, such as UV radiation released by sunlight, IR, and numerous genotoxic chemicals can damage the DNA. In addition, the genome is also threatened with by-products of normal cellular metabolism, such as reactive oxygen species which is derived from oxidative respiration and products of lipid peroxidases (Lindahl *et al.*, 1999). In response to these agents a variety of damages occurs in the DNA such as single and double strand breaks, mismatches, and chemical adducts (Figure 1). Therefore, cell has to develop a multiple repair pathway by forming numerous protein cascade events in order to repair the damage as accurately as possible (Nasmyth *et al.*, 1996).

1. Cell cycle checkpoints

All eukaryotic cells have four different phases in cell cycle: G1-phase, S-phase, G2 and M-phase and one phase outside the cell cycle, G-0 (Figure 2). In mammalian somatic cells the phases are well-defined and represent stages in the life of a cell in which distinct biochemical reactions take place (Sancar *et al.*, 2004). The overall length of the cell cycle in cultured mammalian cells is between 18 to 24 hours. A typical G1 phase lasts 8-10 hours whereas the embryonic cells spend only a few minutes in G1. During G1 phase, the internal and external signals together determine whether the cell undergoes a round of duplication. Once the cell passes the restriction point by following S-phase DNA replication must be completed after that reversal is not possible.

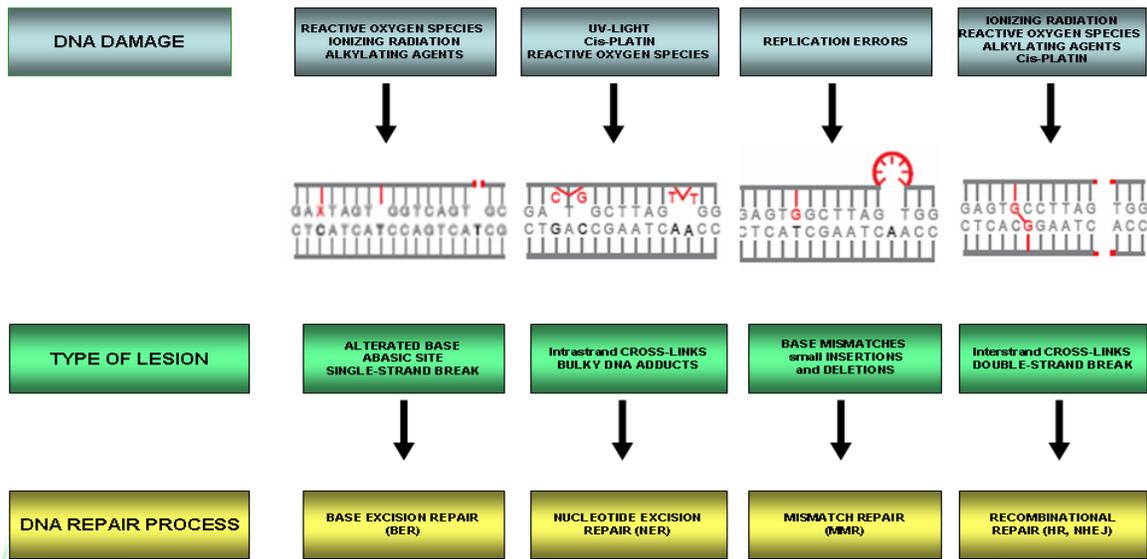


Figure 1: Overview of the most common types of DNA lesions that can be caused by intra and extra cellular agents (Blue boxes). Effect of these agents causes lesion on single strand or double strand of the DNA showed in Green boxes, and repair pathway that operates on the various lesions is also indicated in Orange boxes.

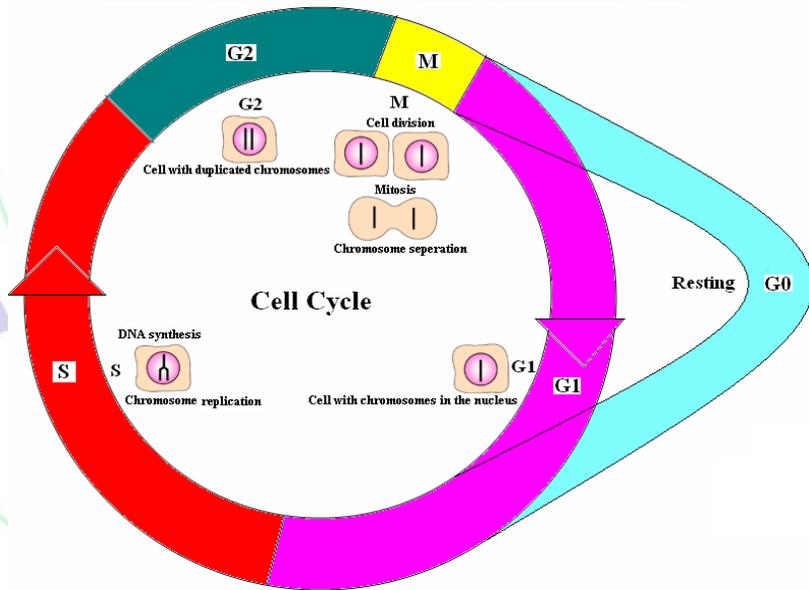


Figure 2: Schematic representation of the cell cycle. DNA damage triggers activation of cell cycle checkpoints, which can lead to an arrest at the G1/S, intra-S, or G2/M. There is a separate region called resting phase G0 also indicated outside the cell cycle.

In the G2-phase, the cell confirms that it is ready to proceed to the subsequent M-phase where the duplicated chromosomes are segregated in less than one hour. If a cell lacks

nutrients in the G1-phase, it enters transiently into a resting G0-phase (Figure 2). However, they may stay in this state for hours, days, weeks or even years before they start dividing

again or even stay in G0 permanently until the organism dies (Becker *et al.*, 2006).

1.1 Molecular component of cell cycle checkpoint

Recent studies in yeast and human cells show that many proteins sense the DNA damage;

these include sensor proteins, signal transducer proteins and effector proteins. These effector proteins launch a cascade of events which mediate cell cycle arrest, apoptosis, DNA repair, and activation of damage induced transcription programs (Table 1).

Table 1: Schematic representation of DNA damage checkpoint protein homologs in different eukaryotes.

Protein function	<i>S.cerevisiae</i>	<i>S.pombe</i>	<i>Xenopus</i>	Mammals
Sensors	Rad24	Rad17	Rad17	Rad17
RFC-like	Ddc1	Rad9	Rad9	Rad9
PCNA-like	Mec3	Hus1	Hus1	Hus1
	Rad17	Rad1	Rad1	Rad1
P13-Kinases (PIKK)	Tel1	Tel1	ATM	ATM
	Mec1	Rad3	ATR	ATR
PIKK binding partner	Ddc2	Rad26	???	ATRIP
Mediators	Rad9	Crb2	???	BRCA1???
	Mrc1	Mrc1	Claspin	Claspin
Effector kinases	Rad53	Cds1	Cds1	Chk2
	Chk1	Chk1	Chk1	Chk1

1.1.1 Sensors and their role in repair mechanism

The first group of proteins that have been found to function as sensors consists of proliferating cell nuclear antigen (PCNA) and replication factor-C (RFC). The candidate DNA damage sensors Rad9, Hus1, and Rad1 form a ring structure (the "9-1-1" complex) that can encircle the DNA similar to PCNA (Melo and Toczyski, 2002). Upon DNA damage both of these protein rings load on DNA for instance, during replication PCNA is loaded on the DNA by RFC complex which consists of five subunits (RFC₁₋₅) and the 9-1-1 complex is loaded on damaged DNA by a protein complex that consists of four subunits (RFC₂₋₅) and Rad17, forming the Rad17-RFC₂₋₅ complexes. As soon as the 9-1-1 complex is bound to the damaged DNA, it is expected to form a scaffold for downstream

checkpoint and repair proteins. One of the recent recognized sensor protein complexes that regulates DNA damage repair is MRE11 complex consist of meiotic recombination 11 (MRE11), Rad50, Nijmegen Breakage Syndrome 1 (NBS1) (Petri and Stracker, 2003). Recent studies on the structure and function of MRE11 complex have suggested that this complex protein plays an important role in downstream target for DSB (Houtgraaf *et al.*, 2006).

1.1.2 Signal transducers

Signal transducers are another group of protein players in the early response of DNA damage. They consist of ataxia telangiectasa (AT) mutated AT (ATM) and Rad3 related AT (ATR) (Figure 3). Studies have shown that mutation in

ATM causes an autosomal recessive disorder, characterized by immunodeficiency, neurological disorders, and high cancer predisposition (Shiloh *et al.*, 1997). Electron microscopy has revealed that ATM binds to DNA in monomeric form and upon exposure to ionizing radiation ATM phosphorylates many proteins, including Chk2, p53, NBS1, and BRCA1 (Cortez *et al.*, 1999). ATR was identified later on the basis of sequence and functional homology to ATM (Cimprich *et al.*, 1996). Results from de Klein *et al.* (2000) show that knockout of ATR in mice resulted in embryonic lethality and mutations that caused loss of ATR activity in humans have been associated with the human autosomal recessive disorder, Seckel syndrome. These suggest that ATR also functions in essential cellular processes in damaged cells like DNA replication and cellular differentiation.

mostly cell cycle specific and associate with damage sensors, signal transducers and effectors at particular phases of the cell cycle and as a consequence of these cascade events result in signal transduction specificity. ATM and ATR phosphorylates most of these mediators, the well known mediators are p53 binding protein -53bp (Wang *et al.*, 2003), the topoisomerase binding protein - TopBP1 (Yamane *et al.*, 2002), and mediator of DNA damage checkpoint-MDC1 (Stewart *et al.*, 2003). Additional mediators function on cell cycle checkpoint pathways are breast cancer 1 (BRCA1) and the earlier mentioned Mre11 complex which are also involved in DNA repair (Figure 3). Similarly, the human Claspin which has sequence homology to yeast Mrc1 was originally thought to be a mediator. Based on recent studies conducted with *Xenopus laevis*, Claspin is an important protein required for Chk1 phosphorylation by ATR and it appears to function more like a sensor (Kumagai *et al.*, 2000, 2003).

1.1.3. Mediators

In addition to sensors, signal transducers, effector proteins and many other proteins are involved in the DNA damage response. They are

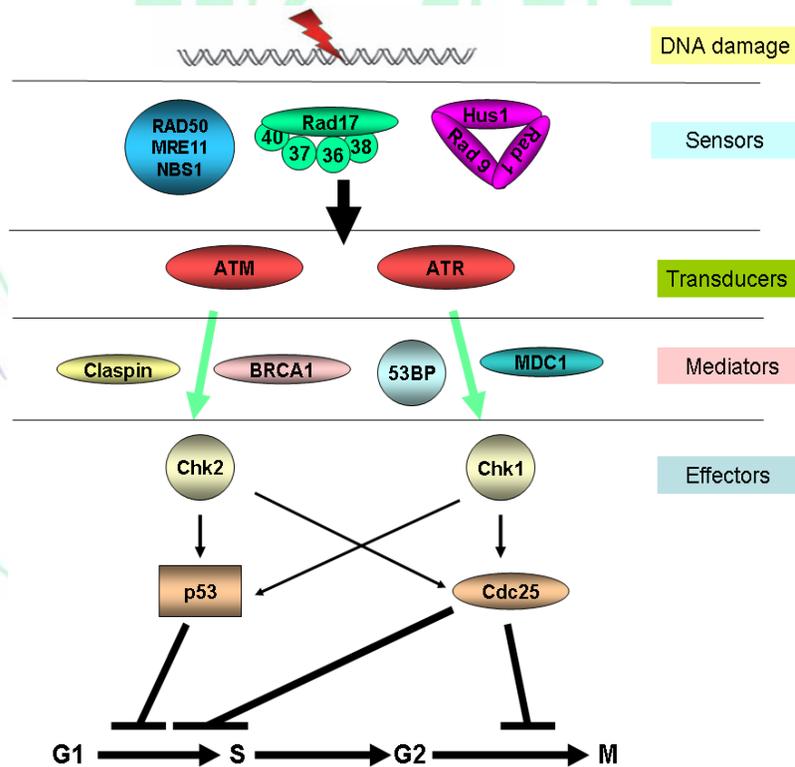


Figure 3: Diagram representing the general outline of the DNA damage response signaling pathway. In response to DNA damage, sensor proteins are activated with aid of mediators, which transmit the signal to transducer proteins. Finally, effectors are activated by the signal transducers. The pathway can lead to cell cycle arrest, transcription or DNA repair. In case of severe damage, apoptosis is induced.

1.1.4. Effectors

The cell cycle checkpoint kinases Chk1 and Chk2 are two serine/threonine protein kinases (Figure 3), which target the downstream effectors of the checkpoint pathways like ATM and ATR (McGowan, 2002). In mammalian cells, both kinases play a key role in all checkpoint pathways responsive to DNA damage. The double-strand break by ionizing radiation signal sensed by ATM is transduced by Chk2 (Matsuoka *et al.*, 2000) and the UV damage signal sensed by ATR is transduced by Chk1 (Abraham *et al.*, 2001).

Moreover, both Chk1 and Chk2 phosphorylate have many common substrates thereby delaying or arresting the cell cycle specific stages (Walworth, 2000). In cell cycle checkpoint, one of the major targets for Chk1 and Chk2 is cell division cycle protein Cdc25, which dephosphorylates and activates Cdk2 (Bartek and Lukas, 2003). Functional role of Chk1 and Chk2 shows that knockout out Chk1 (-/-) causes embryonic lethality in mice (Liu *et al.*, 2000), whereas Chk2 (-/-) knockout mice are viable and appear to exhibit near-normal checkpoint responses (Jack *et al.*, 2002). Another investigation by Bell *et al.* (1999) shows that mutations in human Chk2 gene cause Li-Fraumeni-like cancer-prone syndrome, a genetic disorder that can lead to several types of cancer.

2. G1/S checkpoint

G1 cell cycle checkpoint is one of the best checkpoints in mammalian cells because it prevents the damaged DNA from being replicated by activating the checkpoint kinases. In G1, this whole process prevents the entry of damaged DNA into S phase. Figure 4 shows the two successive cell cycle checkpoint responses at G1/S which depends on the kind of DNA damage. For instance, if the DNA damage is double-strand breaks caused by ionizing radiation, which leads to phosphorylation of ATM that subsequently phosphorylates Chk2. Single-strand gaps result in the activation of Rad17-RFC, the 9-1-1 complex, and ATR, which leads to phosphorylation of Chk1.

DNA damage response operates via post-translational modification, which activates both Chk1 and Chk2 kinases, which further phosphorylate the Cdc25A phosphates (Lukas

and Bartek, 2003). Cdc25A phosphorylation is inactivated by nuclear exclusion and ubiquitin mediated proteolysis (Busino *et al.*, 2004). Study from Falck (2001) demonstrated that the lack of Cdc25A leads to addition of the phosphorylated form of Cdk2. Phosphorylation of Cdk2 inhibits the loading of Cdc45 onto chromatin and thereby prevents the initiation of DNA replication (Falck *et al.*, 2001).

In contrast to Cdc25A, G1/S arrest is also regulated by p53. Both ATM and ATR phosphorylate p53 directly on Ser15 and indirectly on Ser20 via activation by phosphorylation of Chk2 and Chk1 respectively (Abraham, 2001). The phosphorylation of p53 inhibits its nuclear export resulting in increased levels of p53 (Zhang *et al.*, 2001). The activation of p53 increases the transcription of p21, the inhibitor of cyclin-dependent kinases, CDKs (Ryan *et al.*, 2001). p21 (WAF-1/Cip1) also binds to the Cdk4-CyclinD complex and prevents it from phosphorylating Rb (Harper *et al.*, 1993). In its unphosphorylated form, Rb binds the E2F transcription factor of S-phase genes and thereby prevents the transition to S-phase (Harbour and Dean, 2000). In case of the continued existence of DNA damage in G1, p53 can induce apoptosis or the cell can enter senescence (Kohn and Pommier, 2005).

3. Intra-S phase checkpoint

The intra-S phase checkpoint comprises a large set of checkpoint and repair proteins. During DNA replication and repair processes in S-phase, cell either repair the DNA damage before the DNA polymerase encounters the DNA lesion, or the cell may allow bypass of the lesion and restore the DNA later. In the former state, DNA replication will slow down because of the DNA repair and in the latter state cell cycle arrest will allow the DNA repair.

The damage sensors for the intra-S checkpoint encompass a large set of checkpoint and repair proteins. It has been shown that when the damage is double-strand break or a double-strand break resulting from the replication of a nicked or gapped DNA the ATM M/R/N complex and BRCA1 are required for the activation of the checkpoint (Howlett *et al.*, 2002). Two pathways are mediated when the S-phase checkpoint is initiated by double-strand breaks.

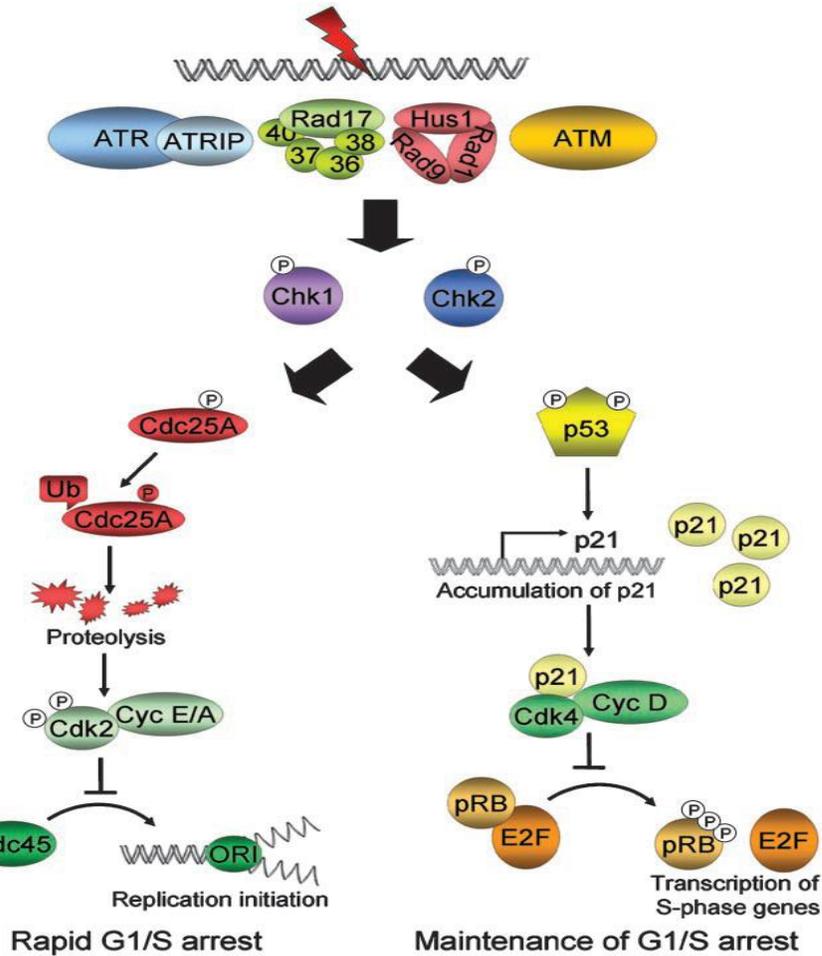


Figure 4: The G1/S checkpoint. Transient and rapid G1/S arrest is achieved by post-translational modification and maintenance of G1/S via p53, E2F, and pRb (adopted from Sancar *et al.*, 2004).

Firstly, the well understood ATM-Chk2-Cdc25A-Cdk2 pathway undergoes a checkpoint response. In S-phase this pathway delays replication by blocking the loading of Cdc45 onto chromatin that in turn attracts DNA polymerase- α as a consequence it extends the DNA replication time allowing DNA repair to take place. The second pathway of S-phase checkpoint is independent of Cdc25A (Figure 5), but requires the activities of ATM which expressed one or more inactive dimers that undergo autophosphorylation (Bakkenist *et al.*, 2003). Activated ATM rapidly phosphorylates the C-terminal tail of histone H2A variant H2AX tagging DNA double-strand breaks and forming the initial signal for the subsequent migration of other checkpoint proteins (Stucki *et al.*, 2005).

The localization of MDC1 with H2AX is necessary for the recruitment of 53BP1, BRCA1 and the Mre11-NBS1-Rad50 complex. ATM-dependent phosphorylation of BRCA1, NBS1 and two additional players in the NBS1 branch of the pathway the SMC1, a chromosomal structural maintenance protein and FANCD2, a Fanconi anemia complementation group D2 protein, both are needed for an intact intra-S phase checkpoint (Shiloh *et al.*, 2003; Andrea *et al.*, 2003). Mutation in NBS1 gene results in Nijmegen breakage syndrome. As mention earlier, NSB1 forms a complex with Mre11 and Rad50 along with H2AX at DNA damage sites. Formation of this complex structure results in DNA double-strand break repair by HR (Sakamoto *et al.*, 2007).

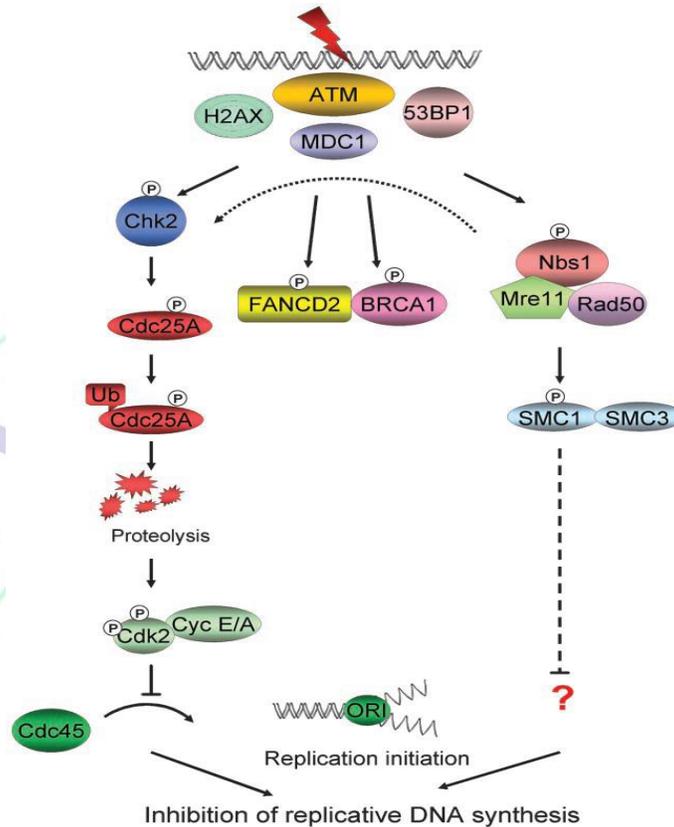


Figure 5: Representation of intra S-phase checkpoint. In this checkpoint, molecular mechanism of the intra S-phase checkpoint shows, in contrast to G1/S and G2/M transitions, S phase can only be delayed, and never permanently blocked in the presence of DNA DSBs generated by ionizing radiation (Sancar *et al.*, 2004).

4. G2/M checkpoint

In G2/M checkpoint, if the cell bypasses S-phase with damaged DNA, the G2/M checkpoint stops the cell cycle in order to prevent the cell from entering mitosis. As in previous G1/S checkpoint the kind of DNA damage determines the pathways. In G2/M checkpoint DNA damage caused by IR induced double-strand breaks which activate the ATM-Chk2-Cdc25 pathway. DNA damage created by UV light activates ATR-Chk1-Cdc25 pathway (Figure 6) to arrest the cell cycle (Donzelli *et al.*, 2003; Sancar *et al.*, 2004). In any event, checkpoint kinases inhibit the entry into mitosis by down-regulating Cdc25 and up-regulating Wee1, which together control Cdc2/CyclinB activity (Boutros *et al.* 2006). Initially, it was thought that Cdc25C is the effector of G2/M checkpoint (Peng *et al.*, 1997). Chen *et al.* (2001) found that mouse Cdc25C knockout cells have a normal G2/M checkpoint. In contrast, disruption of the Chk1-Cdc25A pathway by withdrawing ionization-radiation

induced S and G2 checkpoint, indicating that Cdc25A phosphatase is the main effector of the G2/M checkpoint (Zhao *et al.*, 2002). Upon phosphorylation, the Cdc25 phosphatase binds to the 14-3-3 proteins and becomes set apart in the cytoplasm and degraded by the ubiquitin-proteasome pathway (Peng *et al.*, 1997).

In addition to the checkpoint kinases, checkpoint mediators such as a BRCA1, 53BP1, and MDC1 are involved in the G2/M checkpoint. On the other hand p53, a tumor suppressor protein has been shown to participate in the G2/M checkpoint (Stewart *et al.* 2003), just as in the G1/S checkpoint. P53 is involved in maintenance rather than in the initiation of G2 cell cycle arrest. For instance, wild-type p53 HCT116 cells were able to sustain a G2 arrest in response to IR. Whereas, the isogenic p53-null derivative (copied) arrested initially in G2 but then escaped and entered mitosis (Bunz *et al.*, 1998). Further study of p53 has to be carried out in the G2/M checkpoint in response to DNA damage by IR and UV respectively.

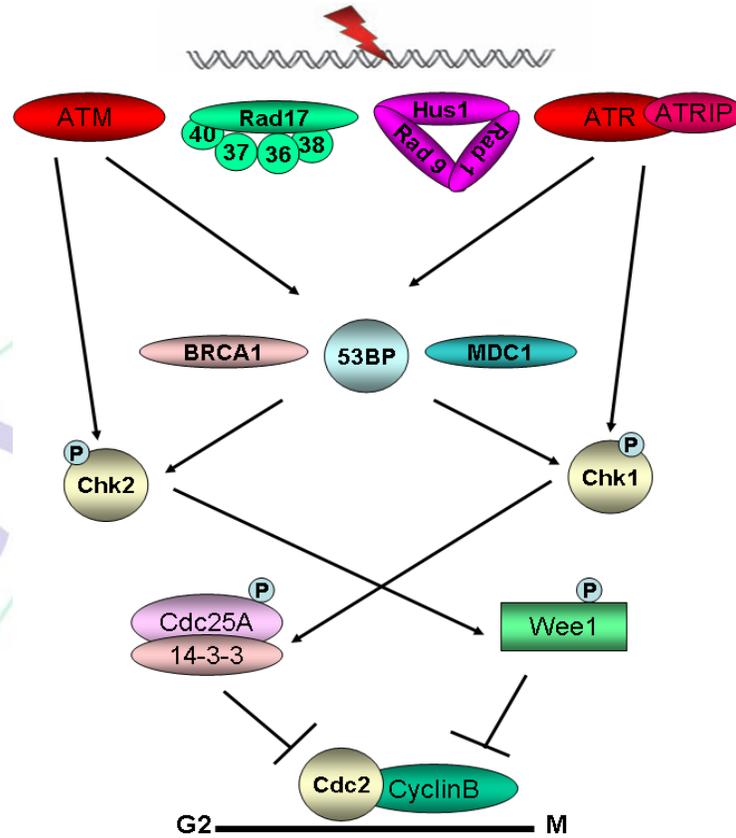


Figure 6: The G2/M checkpoint. The ionizing radiation (ATM) and UV damage responsive sensor proteins (ATR-ATRIP, Rad17-RFC, and 9-1-1) are recruited to the damage site. The mediator proteins such as MDC1, BRCA1 and/or 53BP1 communicate the DNA damage and signal to Chk1 and/or Chk2, thereby regulating the Cdc2/CyclinB, Wee1, and Cdc25A proteins that are crucial for the G2/M transition by changing their expression, phosphorylation, and cellular localization.

5. DNA repair mechanisms

DNA damage checkpoints which involved in a cascade of proteins that can only prevent the transduction of mutations to daughter cells by means of efficient DNA damage repair machinery. As there are many different lesions possible, DNA repair pathways can be divided into five categories:

- (i) direct repair,
- (ii) base excision repair (BES),
- (iii) nucleotide excision repair (NER),
- (iv) mismatch repair (MMR),
- (v) double-strand break repair (DSBR).

(i) Direct repair

Ultraviolet radiation range of 200-300 nanometers is harmful to the biological functions of DNA. The action of UV-irradiation results in

the formation of cyclobutane thymine dimers which are the major photoproducts in the DNA damage (Heelis *et al.*, 1993). Most of the organisms have two direct repair mechanisms: The most direct form of DNA repair mechanism is the photo-reactivation of UV-induced pyrimidine dimers by DNA photolyase. Another form of direct repair mechanism is by removal of the methyl group from DNA by O (6)-methylguanine-DNA methyltransferase. It is important to note that photolyase is not present in many species including humans, whereas methylguanine DNA methyltransferase has nearly universal distribution in nature (Sancar *et al.*, 1994).

(i-a) *Photolyase* is a monomeric protein of 55-65 kDa with two chromophore cofactors, a pterin in the form of 5-10-methenyltetrahydrofolate

(MTHF) and a flavin in the form of FADH⁻ (Sancar *et al.*, 1994). Current data supports the following major steps in the photorepair mechanism. The photolyase enzyme binds to the dimer in a (UV-visible) photon and the MTHF cofactor absorbs a blue light photon and then excites FADH⁻ by energy transfer (Figure 7). The excited FADH⁻ transfers an electron to split the dimer and the electron is transferred back to photolyase to regenerate FADH⁻ and the enzyme dissociate from the repaired DNA.

(i-b) *O6-methylguanine-DNA-methyltransferase* (MGMT), also called *O6-alkylguanine DNA alkyltransferase*, is a DNA repair protein of about 20 kDa that does not contain a cofactor and is ubiquitously found in prokaryotic and eukaryotic organisms (Figure 8) (Sancar *et al.*, 2004). The MGMT enzyme removes mutagenic and cytogenic adducts from *O6*-guanine in DNA, via a one-step methyl transfer reaction (Lindahl *et al.*, 1988). During this alkylation process on DNA, the methyl group is transferred from alkylated base onto a cysteine residue in the active site of MGMT by blocking DNA replication (Erickson *et al.*, 1980). The inactivated alkyl-MGMT protein is then degraded in an ATP-dependent ubiquitin proteolytic pathway (Srivenugopal *et al.*, 1996). MGMT has been shown to prevent mutations in a number of target genes including the p53 genes (Hussain *et al.*, 1994) and the Ras oncogene (Esteller *et al.*, 2000).

(ii) Base excision repair

Base excision repair is a multi-step process that corrects non-bulky damage to bases resulting from oxidation, methylation, deamination, or spontaneous loss of the DNA base itself (Memisoglu *et al.*, 2000). A recent study has identified the importance of this pathway by inactivating the BER core proteins in mice leads to embryonic lethality (Houtgraaf *et al.*, 2006). BER has two subpathways: both of these pathways initiated by the action of a DNA glycosylase that cleaves the N-glycosidic bond between the damaged base and the sugar phosphate backbone of the DNA (Figure 9). This cleavage generates an apyrimidinic/apurinic (AP) in the DNA which is substrate for the single-patch repair pathway. In the alternative long-patch repair, AP endonuclease (APE) cuts the sugar phosphate backbone 5' to the AP site (Sancar *et al.*, 2004). The single-patch, one-nucleotide gap is filled in by DNA polymerase- β (Pol β) and subsequently ligated by the ligase3/XRCC1 complex. Unlike single-patch repair, the long-patch repair is PCNA-dependent pathway (Frosina, 1996) in which the involvement of DNA polymerase- β and DNA polymerase δ/ϵ as well as the FEN1 endonuclease adds several nucleotide to remove the displaced DNA flap and DNA ligase1 for sealing the backbone of the DNA double helix.

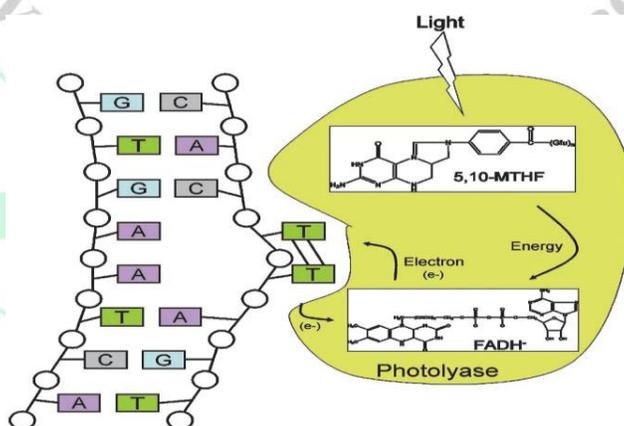


Figure 7: Structure showing the interaction of photolyase with pyrimidine dimer to split and repair the DNA.

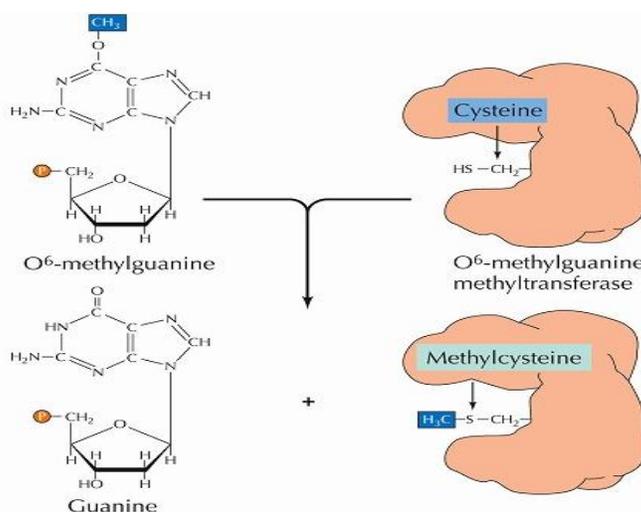


Figure 8: O⁶-methylguanine methyltransferase (MGMT). The MGMT enzyme transfers the methyl group from the O⁶-methylguanine DNA adduct to a cysteine residue in the enzyme and becomes irreversibly inactivated (*Picture taken from the book THE CELL, fourth edition*).

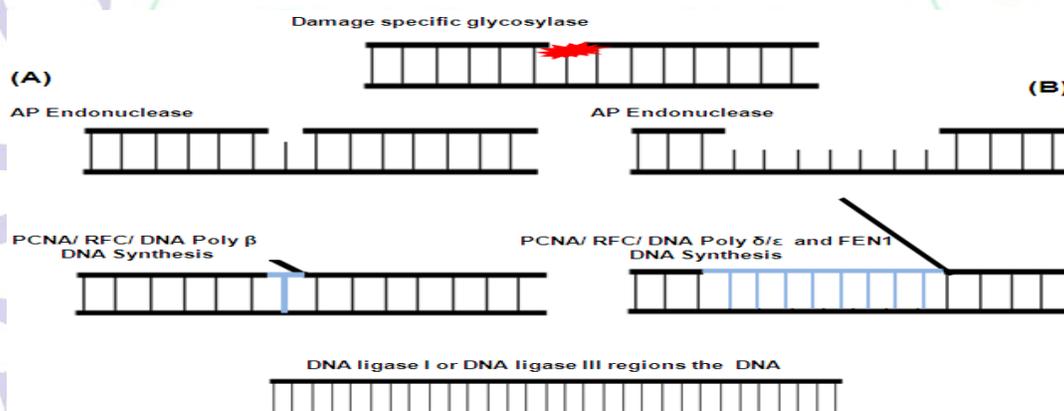


Figure 9: Representation of pathway for base excision repair in human cells. (A) Single-patch repair and (B) long-patch repair pathways: Single-patch repair replaces the lesion with single nucleotide; long patch repair replaces the lesion with approximately 2 to 10 nucleotides. See the main text to understand complete details of this pathway.

(iii) Nucleotide Excision Repair (NER)

NER is the most important repair system to remove bulky DNA lesions that can be caused by UV radiation (Cyclobutane-Pyrimidine-dimers) and 6-4PP (pyrimidine-6-4-pyrimidone photoproducts) other NER substrates include bulky chemical adducts, DNA intra-strand crosslinks, and some forms of oxidative damage are the results of NER (Hess *et al.*, 1997).

The NER mechanism consists of a multistep process (Boer and Hoeijmakers, 2000). Numerous proteins are required for the action of NER that includes damage recognition, local opening of the DNA duplex around the lesion, dual incision of the damaged DNA strand,

gap repair synthesis, and strand ligation (Batty, 2000). In prokaryotes, only three proteins (UvrA, UvrB and UvrC) are required in NER, while more than 30 proteins are involved in the mammalian NER (Truglio *et al.*, 2006). During NER, the damage DNA is recognized by the following binding proteins such as RPA, XPA, and XPC-TFIIH which assemble at the damage site in a random order. These multiple protein repair factors form a complex at the binding site and if the binding site is damage-free ATP hydrolysis by the XPB and XPD helicases dissociate the complex (Figure 10). If the site contains a lesion, ATP hydrolysis unwinds the duplex by about 25 bp around the lesion making a stable pre-

incision complex 1 (PIC1) at the damage site. If the complex binds at damaged site XPG replaces XPC and making more stable pre-precision complex 2 (PIC2). Finally, XPF.ERCC1 is recruited to the damage site to form pre-precision complex 3 (PIC3). The endonuclease XPG and ERCC1/ XPF then cleave one strand of the DNA at positions 3' and 5' by generating an approximately 24-32 base oligonucleotide. The resulting 24-32 oligonucleotide is released and the gap is filled by DNA polymerase δ/ϵ with the aid of replication accessory proteins PCNA and RFC.

Knowledge of human NER has been gained through the study of two human genetic

syndromes, Xeroderma pigmentosum (XP) (Petit and Sancar, 1999) and Cockayne syndrome (CS) (Hanawalt *et al.*, 2002). These diseases are characterized by neuro-degeneration resulting in increased cancer frequency and ageing. XP is caused by defect in one of two genes XPA to XPG whereas, CS is caused by defect in one of two genes (CSA or CSB) (Thoms *et al.*, 2007). The XP gene products are now known to perform various functions during damage recognition and DNA incision (Lindahl, 1999). On the other hand, the CS gene products are required for NER-based repair of transcriptionally active genes (Friedberg, 1996).

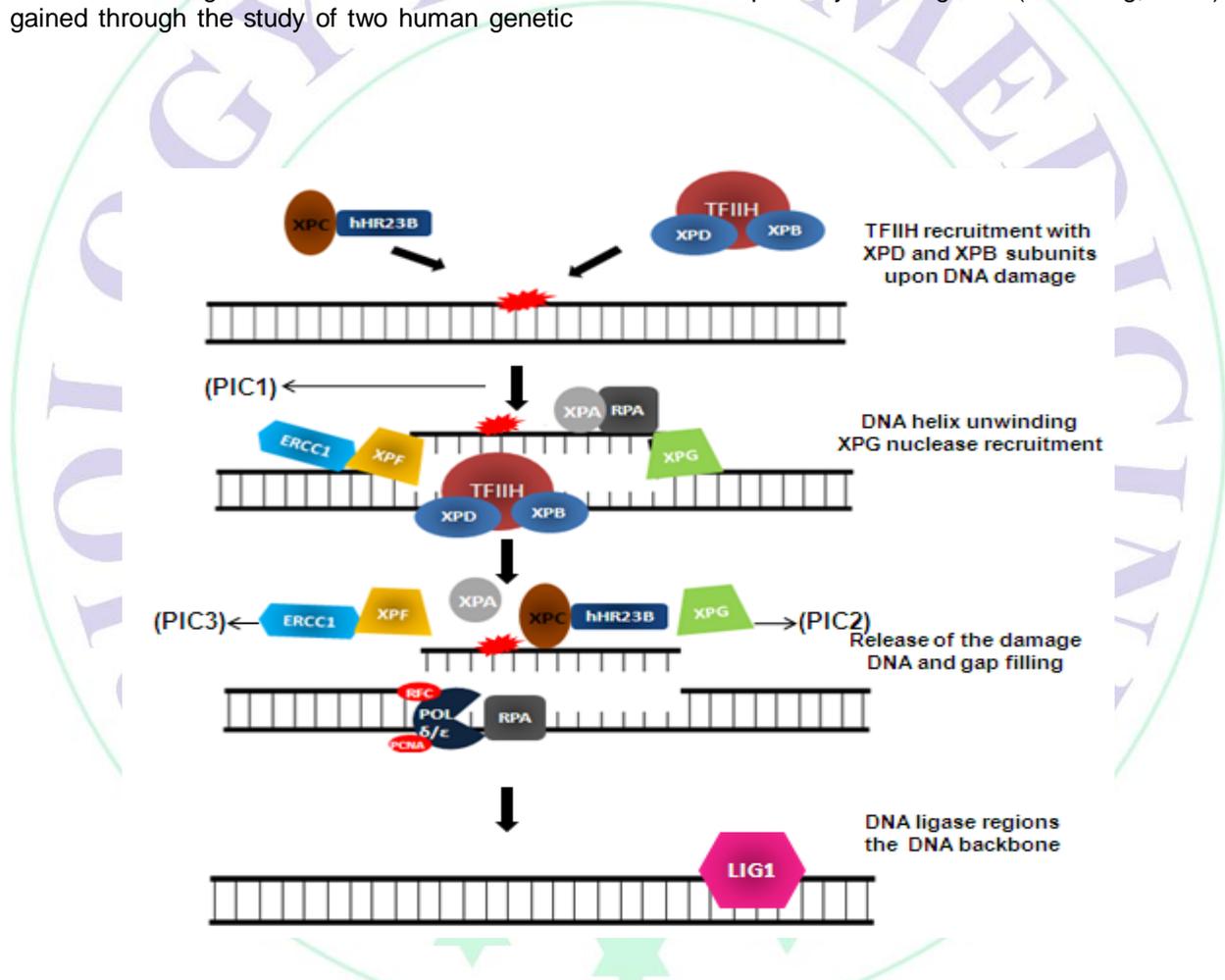


Figure 10: A simplified model of Nucleotide Excision Repair (NER) pathway is shown. DNA damage recognition by the interaction of RPA, XPA, and XPC-TFIIH, which assemble at the damage site. Damage site is ATP dependent, if the site found lesion, ATP hydrolysis unwinds the duplex by about 25 bp around the lesion, making a stable pre-precision complex 1 (PIC1) at the damage site. XPG then replaces XPC in the complex to form a more stable pre-precision complex 2 (PIC2). Finally, XPF-ERCC1 is recruited to the damage site to form pre-precision complex 3 (PIC3). The resulting 24-32 oligomer is released, and the gap is filled by pol δ/ϵ with the aid of replication proteins PCNA and RFC.

(iv) Mismatch repair

Mismatch repair (MMR) systems is a multistep process that play an important role in promoting genetic stability by repairing DNA replication errors such as, base-base mismatches (A-G or C-T) and insertion /deletion loops (IDLs) that result from DNA polymerase misincorporation of nucleotide and template slippage, respectively (Harfe and Jinks-Robertson, 2000). DNA MMR proteins were first derived from studies of *E. coli* and it shows that MMR proteins are highly conserved process from prokaryotes to eukaryotes (Obmolova *et al.*, 2000). For instance, there are three major MMR proteins constituted *in vitro*: MutS, MutL, and MutH. MutS is an ATPase dependent protein that homodimerizes the base/base mismatch or insertion/deletion loops (IDLs).

Unlike bacterial MMR system, the mammalian MMR system contains proteins related to the bacterial MutS and MutL proteins but is more complex than the bacterial system. It involves two different heterodimeric complexes of MutS-related proteins, MSH2-MSH6 (known as MutS α) and MSH2-MSH3 (known as MutS β), and each has different mispair recognition specificity (Genschel *et al.*, 1998) (Figure 11). However, the MSH2-MSH6 focuses on the recognition of heterodimers single base-base mismatches and small insertion/deletion loops (IDLs). Whereas, MSH2-MSH3 recognizes the

larger loops (2-10 bases). Most (80-90%) of the MSH2 in the cell is complexed with MSH6 to form MutS α . Thus, MutS β is a relatively minor activity. Similarly, instead of a single MutL-related protein, mammalian MMR involves a heterodimeric complex of two MutL-related proteins, MLH1-PMS1 (PMS2 in humans) (Kolodner *et al.*, 2002). Heterodimeric complexes of MLH1/PMS2 (MutL α) and MLH1/PMS1 (MutL β) interact with MSH complexes and replication factors (Buermeier *et al.*, 1999). Excision and re-synthesis of the nascent strand (containing the mismatch or IDL) is performed by a number of proteins including PCNA (Proliferating Cell Nuclear Antigen), RPA (Replication Protein-A), RFC (Replication Factor-C) Exonuclease-I, DNA Polymerases Delta/Epsilon, Endonuclease FEN1 (Flap structure-specific Endonuclease-1), and additional factors (Buermeier *et al.*, 1999). According to studies published, defects in MMR genes cause a predisposition to hereditary non-polyposis colorectal carcinoma (HNPCC) (Fishel *et al.*, 1993). Patient affected with HNPCC tumor shows the occurrence of instability of simple repetitive DNA sequences (microsatellite instability or MSI). Statistical analysis has shown that more than 90% of the colorectal tumors and at least 75% of the endometrial tumors from HNPCC patients display MSI (Leeuw *et al.*, 2000).

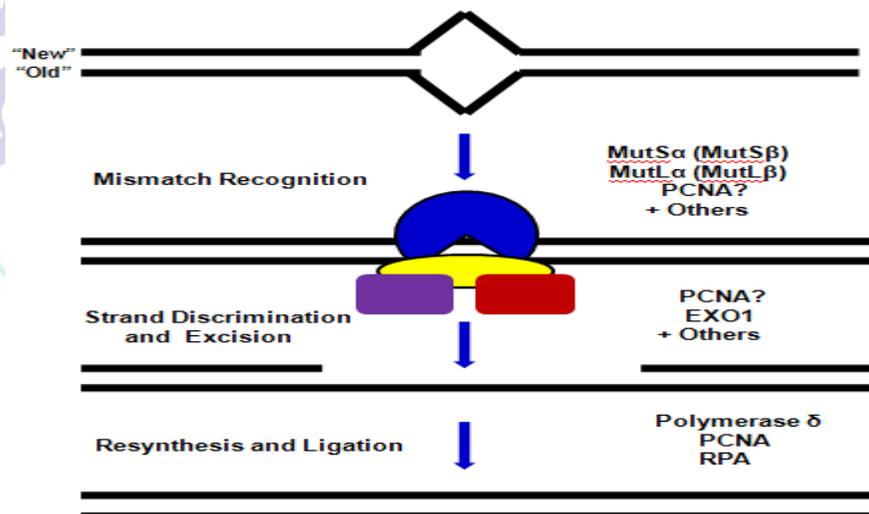


Figure 11: A model representing the mammalian mismatch repair proteins, which shows the following excision of the newly synthesized strand containing the mismatched base or insertion/deletion loop (IDL), strand re-synthesis is performed to restore the fidelity of the DNA.

(v) Double strand breaks

As previously mentioned, IR induces a large variety of DNA lesions especially double strand breaks are considered to be the most lethal lesions (Bradley and Kohn, 1979). DSBs are repaired by two pathways: the so-called homologous recombination (HR) or non-homologous end-joining (NHEJ) (Figure 12) (Kanaar *et al.*, 1998). Vertebrates cells preferentially repair DSBs by HR in the late-S and G2 phases of the cell cycle when an undamaged sister chromatid is available, while NHEJ appears to be confined mainly to the G1/early-S phases (Takata *et al.*, 1998).

(v-a) Homologous recombination of DSB

Homologous recombination repair of DSBs is essential for making error-free genetic information from a homologous undamaged DNA molecule. The majority of HR repair is mediated by Rad52 epistasis group that includes the Rad51, Rad52, Rad54, Rad55, Rad57, MRE11 and NBS1 function in the initial sensor of the broken DNA ends (Sonoda *et al.*, 2001). Processing of the damaged ends ensue the production of 3' ssDNA overhangs. Strand invasion and branch migration is initiated by Rad51 in eukaryotes (Sung, 1994), or RecA in prokaryotes (Cox *et al.*, 2002). Other proteins including RPA (Replication Protein-A), Rad52, Rad54, BRCA1, BRCA2 (Breast Cancer Susceptibility Proteins) and several additional Rad51-related proteins serve as accessory factors in filament assembly and subsequent Rad51 activities (Jackson, 2002) (Figure 12). The Rad51 nucleoprotein filament then searches the undamaged DNA on the sister chromatid for a homologous repair template. Upon identification of homologous template, damaged DNA strand invades the undamaged DNA duplex by a process called DNA strand exchange. A DNA polymerase then extends the 3' end of the invading strand and subsequent ligation by DNA ligase-I yields a hetero-duplexed DNA structure. This recombination intermediate is resolved and the precise error-free correction of the DSB is complete.

(v-b) Non-homologous end-joining of DSB

Non-homologous end-joining (NHEJ) is the most preferred repair mechanism of DSB repair in mammalian cells (Jeggo, 1998). In contrast to HR, this pathway does not require homology and can rejoin broken DNA ends directly end-to-end. The activity of the Ku70/Ku80 heterodimeric protein is essential to the NHEJ

pathway. The Ku heterodimer initiates NHEJ by binding to the free DNA ends and recruiting other NHEJ factors such as DNA-Pk (DNA-dependent protein kinase), XRCC4 to XRCC7 and DNA Ligase-IV (Figure 12). In addition to the Ku and Ligase-IV homologs, the Rad50, MRE11 and NBS1 genes are also involved in NHEJ (Huang *et al.*, 2002). DNA-Pk becomes activated upon DNA binding, and phosphorylation of these factors facilitates the repair process.

6. Role of BRCA1-2 and RAD51 in response to DNA damage

Epidemiological studies show that breast cancer is the most common cancer among women worldwide with a yearly incidence of over 1 million (Mcperson *et al.*, 2000). Breast cancer type-1 and 2 (BRCA1) and (BRCA2) are normal genes carried by all men and women; when functioning properly, these genes help to prevent cells from growing out of control. In this article, we summarize some of the structural and functional role of BRCA1-2 and Rad51 in response to DNA damage.

6.1 The BRCA1 gene

The genetic identification of breast cancer gene has become established over the past two decades with the cloning of two major breast cancer genes BRCA1 and BRCA2 (Wooster *et al.*, 1995). In 1994, BRCA1 gene was cloned and mapped on chromosome 17q21 by genetic linkage analysis. BRCA1 gene encodes a protein which consists of 1863 amino acid sequence with approximately 208 kDa and is expressed in most proliferating cells (Figure 13-A). The COOH-terminus of BRCA1 protein contains an amino acid sequence motif known as BRCT domain. The BRCT domain in the COOH-terminus of BRCA1 seems to be an important protein interaction domain for many DNA repair proteins. It interacts with the BRCA1-associated RING finger domain protein (BARD1) (Jensen *et al.*, 1998) which is required for ubiquitin ligase activity, the MRE11/RAD50/NBS1 (M/R/N) recombination repair complex, a putative DNA helicase BRCA1-associated C-terminal Helicase (BACH1) (Cantor *et al.*, 2001), the CtBp-interacting protein CtIP (Kaelin *et al.*, 1990) and RNA polymerase II (Scully *et al.*, 2000). In the NH-terminus, there is a ring domain for protein-protein interaction but this domain is also involved in protein ubiquitination.

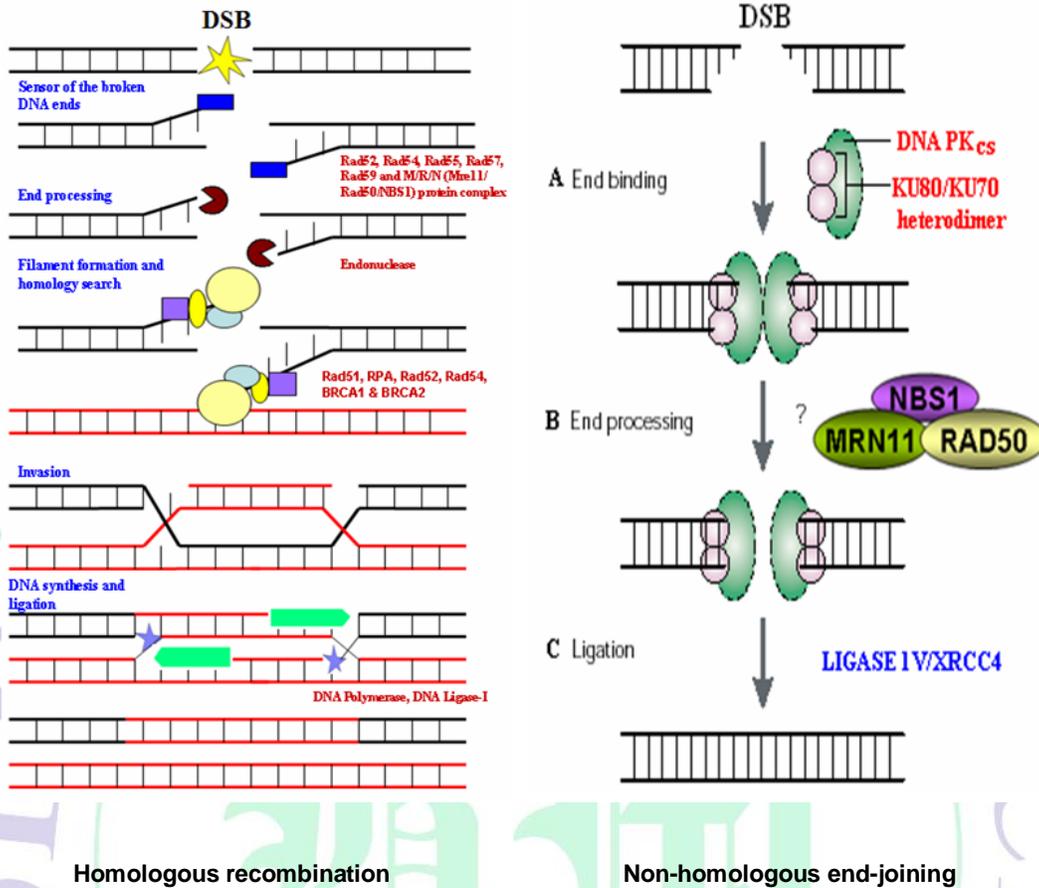


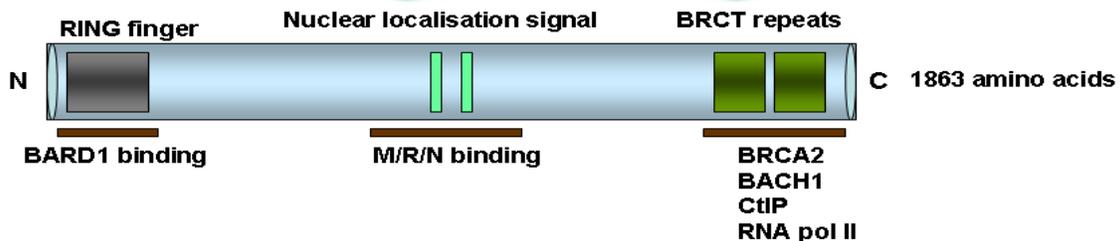
Figure 12: Schematic representation of DSB repair. Overview of the main steps and factors involved in the double strand break repair by homologous recombination are shown on left and non-homologous end-joining are shown on right.

6.2 The BRCA2 gene

BRCA2 gene is very large and mapped on chromosome 13q 12-13. This gene encodes a 3418 amino acid sequence protein which is approximately 385 kDa (Figure 13-B). BRCA2 is characterized by a very large exon11, which encodes peptide motifs required for the interaction with Rad51 protein (Chen *et al.*, 1999). Rad51 is the eukaryotic homology of the prokaryotic recombination protein ReCA and is located at chromosome position 15q15.1 (Takahashi *et al.*, 1994). Rad51 proteins co-

localize with Rad52 (Essers *et al.*, 2002), Rad54 (Essers *et al.*, 2002), the single-strand binding protein RPA (Raderschall *et al.*, 1999) and the tumor suppressor proteins BRCA1 and BRCA2 as mentioned above. BRCA2 interacts with the histone acetylase P/CAF (Scully and Livingston, 2000) BRCA2-associated factor BRAF35 and DSS1. The sites of the eight BRC repeats, six of which interact with Rad51, the oligonucleotide binding (OB) domain that interacts with single-stranded DNA and the carboxyterminal NLS (nuclear localization signal) are indicated.

(A) BRCA1:



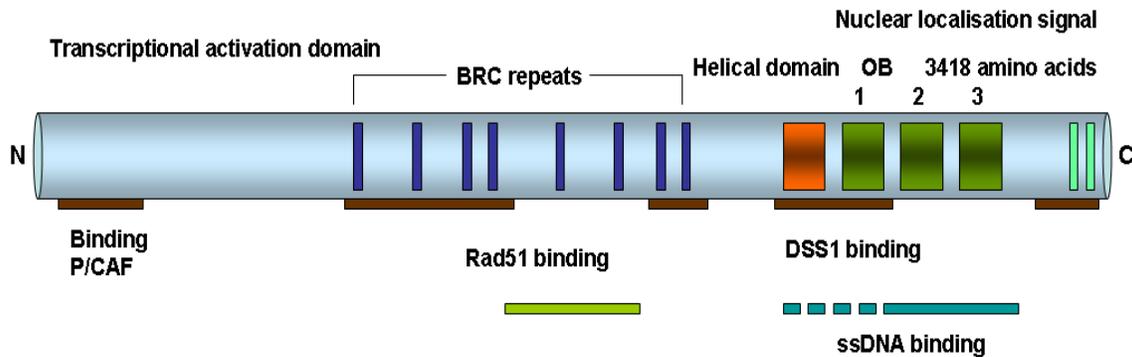
(B) BRCA2:

Figure 13: Schematic structure of **(A)** BRCA1 and **(B)** BRCA2, showing the functional domain of both proteins.

6.3 BRCA2 regulates Rad51 to mediate recombination

BRCA2 plays an important role in the regulating of DSBs repair by homologous recombination, a pathway which is dependent upon the recombinase function of Rad51 (Moynahan *et al.*, 2001). It has been shown that there is a direct interaction between BRCA2 and Rad51 and their co-localization in nuclear foci after DNA damage. This represents the role of BRCA2 within this DNA repair pathway (Chen *et al.*, 1998). Yuan *et al.* (1999) showed that cell lines defective in BRCA2 fail to accumulate Rad51 foci after DNA damage treatment. The structural detail shows that BRCA2 has two distinct Rad51 binding domains in which the first domain consists of a set of eight 35-residue motifs located in the central region of the protein, encoded by exon 11 of the BRCA2 gene (Chen *et al.*, 1998). BRCA2 also binds Rad51 via a C-terminal motif that is unrelated to the BRC repeats and encoded within exon 27 (Christopher *et al.*, 2007).

BRCA2 contains eight BRC repeats in which BRC3 and BRC4 have the strongest interaction with Rad51 with the exception of BRC5 and BRC6 (Bignell *et al.*, 1997). Pellegrini (2002) showed that the crystal structure of the BRC4 peptide sequence (amino acids 1517-1551) of BRCA2 is bound to Rad51. This structure demonstrates a series of hydrophobic and hydrophilic interactions involving the hairpin structure of BRC4 created by the short anti-parallel beta-sheets and alpha-helix. *In vitro* study had suggested that BRC3 or BRC4 peptides could inhibit the formation of Rad51 complexes (Davies *et al.*, 2001). However, in living cells the effect of full-length BRCA2 is to promote homologous recombination (Moynahan

et al., 2001). On the other hand, the expression of the BRC4 peptide inhibits BRCA2 homologous recombination (Xia *et al.*, 2001) and the same observation of interaction between Rad51 and BRC3 or BRC4 inhibiting the DNA binding are also observed by Tarsounas *et al.* (2004). The formation of Rad51 foci require BRCA2 and BRCA1 (Yuan *et al.*, 1999), especially after exposure to ionizing radiation.

These observations have created a question: Why does the BRC4 peptide inhibit filament formation but the whole BRCA2 protein appears to promote filament formation? Answer to this question is - the BRC4 region of BRCA2 interacts with Rad51 such that it prevents Rad51 monomer-monomer interaction, which in turn blocks filament formation on DNA (Davies *et al.*, 2001; Pellegrini *et al.*, 2002). In contrast to BRC, the BRCA2 acts as a loading factor for Rad51 on ssDNA by means of its BRC peptide interaction. As the Rad51 bound to BRCA2 finds its position adjacent to a Rad51 monomer on ssDNA, the monomer-monomer interaction becomes favored over the BRCA2 interaction as we know that the Rad51-dependent homologous recombination and Rad51 filament formation in S-phase occurs in the absence of BRCA2. However, the presence of BRCA2 is required to prevent major errors, such as chromosome aberrations, which occur in BRCA2-deficient cells (Patel *et al.*, 1998). In contrast to the interaction of BRCA2, the region encompassing residues 758-1064 in BRCA1 was first reported to be involved in its interaction with Rad51 (Scully *et al.*, 2000). But it remains unclear, however, whether the two proteins can bind directly.

6.4 Rad51 foci

Immunohistochemistry is the good source of identifying nuclear foci in response to DNA damage. Recombination protein machinery are normally found in the nucleus, they are diffused throughout the nucleus and are rapidly re-localized and concentrated into sub-nuclear complex that are microscopically detected as foci. Results from Tarsounas *et al.* (2004) showed the formation of repair foci in response to ionizing radiation when culture cells were analyzed for the presence of Rad51 foci by

using immune-fluorescence staining, approximately 10-15% of the cells were found to contain Rad51 foci (Figure 14, *non-irradiated cells*). These are thought to represent the population of cells in the culture that are undergoing S phase and are actively replicating their DNA. When the treatment of cells was followed by 10 Gy (a unit of ionizing radiation), the number of cells containing Rad51 foci increased to more than 50% (Figure 14, *irradiated cells*).

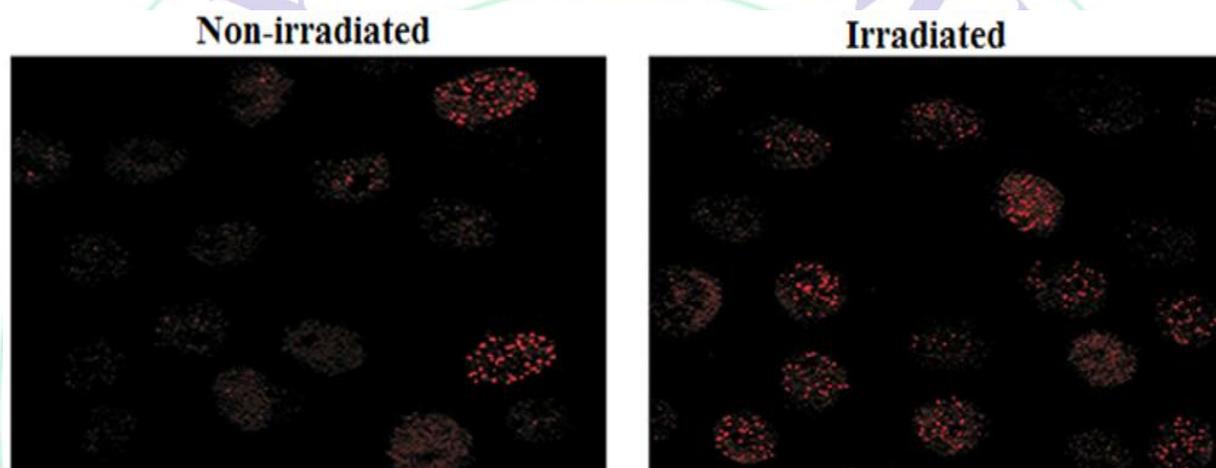


Figure 14: Schematic representation of Rad51 expression after IR exposure. HeLa cells irradiated with 10 Gy and allowed to recover for 3 hours. In comparison to non-irradiated cells, irradiated cells show an increase in the formation of Rad51 foci (Tarsounas *et al.*, 2004).

7. Conclusion

Based on all evidences and observations over the past 30 years, our knowledge about DNA damage, DNA damage checkpoints and DNA repair has increased. In spite of this, there is still a clear need for finding the missing components and connections in the network of the checkpoint signaling. Also, the DNA repair mechanism needs to be analyzed as the concept of DNA damage checkpoint and DNA repair mechanism is very important for our understanding for the treatment of cancer and chemotherapy. More recently, it was reported that a DNA repair biomarkers would provide crucial information on the status of various cancer progression. Therefore, further study is warranted to develop a better functional DNA repair biomarkers for the early detection of quantitative and qualitative analysis for various cancers which could be useful in therapies.

Based on the evidence presented in this review, it is seen that BRCA1/BRCA2 and Rad51 play a central role in homologous recombination. However, the precise role of BRCA1 and BRCA2 as a tumor suppressor is not fully understood. Although, the current knowledge illustrates that cancer cells without a functional BRCA1/2 proteins are resistant to anti-microtubule drug-based chemotherapy, they are sensitive to DNA damaging agents that cause DSBs (ionizing radiation) and inter strand cross-linking agents such as platinum-based drugs (cisplatin and carboplatin). Therefore, it is important to identify the functional role of BRCA1 and BRCA2 proteins which will then be helpful in designing specific drugs in targeting solid tumors.

Understanding the protein-protein interaction also plays a central role in approaching new chemotherapy. Therefore, it is important to know the key role of Rad51

interaction with BRCA2, which acts as a cofactor in homologous recombination. As such, the role of Rad51-dependent homologous recombination constitutes a key function in DNA damage. Based on this interaction, structural models that give a detailed understanding of Rad51 activation and its function can be improved. This might be helpful in improving the design of drugs which can modulate the Rad51 activity and improve the efficacies of radiation therapy and chemotherapy.

Conflict of Interests

None declared.

Acknowledgement

We are thankful to Dr. Aziz Sancar, Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, USA and Dr. Stephen C West (FRS), London Research Institute, Clare Hall Laboratories, UK for giving us the permission to use some of their images for this review article.

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